The effect of modafinil on counter-regulatory and cognitive responses to hypoglycaemia

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Abstract

Aims/hypothesis. Our hypothesis is that reducing release of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) with modafinil will enhance symptomatic and hormonal responses to hypoglycaemia.

Methods. Nine healthy men received, in random order, two 100-mg doses of modafinil or placebo, followed by an insulin clamp in which plasma glucose was either reduced stepwise to 2.4 mmol/l or was sustained at euglycaemia (four studies). Catecholamines, symptom scores and cognitive function were measured.

Results. Modafinil had no effect on the measured parameters during euglycaemia. During hypoglycaemia,

autonomic symptom scores were significantly higher with modafinil (increase at lowest plasma glucose concentration 271.3 ± 118.9 vs $211.2 \pm 80.4/40$ min, $p=0.019$), and the heart rate response was increased ($12,928 \pm 184$ vs 6773 ± 148 bpm/140 min, $p=0.016$). Deterioration in performance of two cognitive tasks was reduced: Stroop colour-word test (613 ± 204 vs $2375 \pm 161/65$ min, $p=0.009$) and accuracy of a simple reaction task (11.3 ± 1.8 vs 9.4 ± 3.7 , $p=0.039$).

Conclusions/interpretation. We conclude that modafinil improves adrenergic sensitivity and some aspects of cognitive function at hypoglycaemia, possibly by reducing neuronal central GABA concentrations.

Keywords Cognitive function · Gamma-aminobutyric acid · Hypoglycaemia · Modafinil

Introduction

The ventromedial nucleus of the hypothalamus (VMH) is important in the sympathetic and neuroendocrine responses to hypoglycaemia [1, 2, 3]. Its glucose-sensing neurones share characteristics with the

pancreatic beta cell, including a specific glucose transporter [4], pancreatic-specific glucokinase [5], intracellular ATP and the K-ATP channel complex [6, 7], and they alter their firing rate in response to changes in glucose concentration [8]. An attractive hypothesis is that brain glucose sensing uses the same cellular mechanisms to control counter-regulation as the beta cell does to control insulin secretion in response to changing plasma glucose concentrations.

Pancreatic beta cells respond to high blood glucose concentration by releasing insulin. We hypothesised that the glucose-sensing neurones of the VMH act in the same way, but releasing a neurotransmitter instead of insulin. The neurotransmitter released must be inhibitory of sympathetic activation, as sympathetic activation occurs in response to low blood glucose. We postulated that the resting state of the VMH was one of tonic inhibition of sympathetic activation, which was de-repressed by hypoglycaemia.

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Abbreviations: GABA, gamma-aminobutyric acid ·
VMH, ventromedial hypothalamus

Gamma-aminobutyric acid (GABA) is a major inhibitory neurotransmitter of the brain [9] and is present in pancreatic beta cells and in the VMH [10, 11]. A subgroup of VMH glucose-sensing neurones has recently been shown to receive synaptic input from GABAergic neurones, potentially located outside of the VMH [12, 13]. The relationship between extracellular brain glucose concentrations and the concentration of GABA is controversial and support for this relationship comes mostly from animal studies. Streptozotocin-induced diabetic rats have high GABA levels, low noradrenaline and reduced sympathetic nerve activity within the VMH [14]. Infusion of glucose into rat substantia nigral neurones increases GABA concentrations, whilst both the perfusion of the glucoprivic agent 2-deoxyglucose and systemic hypoglycaemia significantly reduced GABA in some [15] but not all studies [16, 17]. Perfusion of the neurones with K-ATP channel activators lowered GABA levels, and antagonists increased them, in a manner analogous to the insulin secretory responses to these agents by pancreatic beta cells, demonstrating the close relationship between GABA, intracellular ATP levels, the K-ATP channel complex and glucose sensing.

We postulated that pharmacological suppression of brain GABA concentration may enhance counter-regulatory responses to systemic hypoglycaemia. Modafinil, used in the treatment of narcolepsy, lowers brain GABA concentration. It is a wakefulness-promoting agent that improves vigilance and cognitive performance during sleep deprivation in patients with narcolepsy and in healthy volunteers [18, 19, 20]. We examined whether this agent, with acute administration, enhances counter-regulatory responses to hypoglycaemia in human subjects.

Subjects and methods

Subjects. Nine healthy male volunteers were recruited. They were aged between 21 and 38 years (mean $\pm$ SD 27.2 $\pm$ 6.2 years) with a mean BMI of 22.4 $\pm$ 3.4 kg/m². None of the subjects had any previous significant medical history or was taking medication. The protocol was approved by the King's College Hospital Ethics Committee and each subject gave written informed consent before participating. All nine subjects underwent paired hypoglycaemic-hyperinsulinaemic clamp studies in random order, each at least 2 weeks apart, in which they received either modafinil or placebo prior to clamping in a double-blind fashion. Six subjects underwent additional paired euglycaemic clamp studies in random order, receiving either modafinil or placebo in double-blind manner. There was a period of at least 2 weeks between any two studies.

Study design. The evening before the study the subject received either a 100-mg tablet of modafinil or a placebo tablet, depending on randomisation, to take with food. The following morning, subjects were admitted to the Programmed Investigation Unit in King's College Hospital, having fasted from 22.30 hours on the previous evening. Two intravenous cannulae were

placed in the non-dominant arm using aseptic techniques and 1% intradermal lignocaine to anaesthetise the skin. One cannula was inserted into the antecubital vein for infusion of insulin, glucose and potassium; the other was placed retrogradely into a distal wrist or hand vein. The hand was rested in a box of heated air (55–60 °C) to arterialise venous blood [21]. This cannula was used for sampling arterialised blood. Forty-five minutes before starting the study, the subjects took another 100-mg tablet of modafinil or placebo. No less than 30 min after the cannulae were inserted, and after baseline blood samples were taken, a primed continuous infusion of soluble insulin (human Actrapid; Novo Nordisk Pharmaceuticals, Crawley, UK) was started, with a maintenance rate of 1.5 mU·kg body weight⁻¹·min⁻¹. This insulin dose was used in all studies. Arterialised venous plasma glucose was measured at the bedside every 5 min and controlled by adjusting a simultaneous variable infusion of 20% glucose (Clintec Nutrition, Slough, UK). Additional blood samples were taken at pre-determined times for the later measurement of catecholamines, growth hormone, glucagon, cortisol and insulin.

During the hypoglycaemia studies, plasma glucose levels were maintained at 5 mmol/l for 40 min before being sequentially reduced to 4.4, 3.8, 3.4, 2.8 and 2.4 mmol/l. Levels were maintained at 4.4 and 2.4 mmol/l for 20 min only. Each other level was maintained for 40 min, with the target level being reached during the first 10 to 15 min and being maintained until the end of the given time period. After the nadir, the plasma glucose level was restored to 5 mmol/l and maintained at euglycaemia for 20 min. In the euglycaemic studies, arterialised plasma glucose was maintained at 5 mmol/l throughout.

Cognitive function was assessed by four-choice reaction time, simple reaction time, finger tapping and Stroop colour-word interference. Finger tapping, simple reaction time and four-choice reaction time were measured using a laptop computer (Toshiba T1800 calculator). Subjects were trained for all tasks before each study, except for the Stroop colour-word interference test, and each task was performed at least three times until stable results were obtained. Finger tapping and four-choice reaction time were then measured twice at 5 mmol/l and once at each glucose level, or at the equivalent time point in the euglycaemic studies. Simple reaction time was measured twice at 5 mmol/l, once at 2.4 mmol/l and at recovery.

Cognitive tasks and symptom scoring. For finger tapping, the subject was asked to press a computer key as fast as possible over a 10-second period [22] and the number of taps was counted. This was repeated three times for each test. The mean of the three recordings was used in the analysis. To record four-choice reaction time [22], 500 targets were displayed on the computer screen, one at a time, randomly appearing in one of four quadrants, over a 5-min period. The subject responded to each by pressing the corresponding key on the adapted keyboard. The mean time of reaction and the accuracy of the responses were recorded. For simple reaction time, the screen randomly displayed a series of letters, and the subject was instructed to press the computer spacebar when the letter X appeared, which it did randomly 12 times during the test. Again, the reaction time and the accuracy of the response were measured. In the Stroop colour-word interference test [23, 24] the subject was presented with a sheet of paper on which the names of colours (red, tan, green and blue) were printed in incongruously coloured ink, e.g. the word "red" printed in blue ink. The subject was then asked to name the colour of the ink. The subject could correct himself but there was no prompting. The number of colours read over 45 seconds was recorded, and the number of errors was subtracted from the total score.

Fig. 1. Plasma glucose concentrations during hypoglycaemia with (open circles) and without (filled squares) modafinil. Data are expressed as means $\pm$ SE

Symptoms were assessed using a questionnaire with a linear digital scale, on which subjects graded a standard list of symptoms from 1 (absent) to 7 (severe). Symptom scores were checked four times in the first 60 min and then on two occasions at each plasma glucose level before and after the cognitive test battery. Individual scores were created for all symptoms (total) and individually for both neuroglycopenic symptoms (difficulty speaking, confusion, dizziness, irritability, blurred vision and drowsiness) and autonomic symptoms (sweating, anxiety, tremor, palpitations, feeling hot and tingling) by adding the scores for each relevant symptom at each time point [25]. At the same time points, physiological measurements of heart rate and blood pressure were also recorded.

Measurements. Arterialised plasma glucose was measured at the bedside using a glucose oxidase technique (Yellow Springs glucose analyzer; Yellow Springs Instruments, Yellow Springs, Ohio, USA). Plasma adrenaline and noradrenaline were measured by HPLC with electrochemical detection [26], glucagon [27], cortisol [28], growth hormone [29] and insulin [30] by a radioimmunoassay. Inter- and intra-assay variations were less than 10% for all assays. Studies on one individual were run in the same assay.

Data analysis. Results are expressed as means $\pm$ SD unless otherwise stated. The results of the hypoglycaemia studies were compared with each other, and the results of the euglycaemic studies were examined separately. Changes in counter-regulatory hormones and symptom scores were calculated as the AUC using the trapezoidal rule during hypoglycaemia, correcting for the initial 40-min euglycaemic period in hypoglycaemic studies and for the duration of the clamp in euglycaemic studies. Plasma glucose thresholds during the hypoglycaemic studies were defined as the plasma glucose concentration associated with the onset of a significant change in any parameter, a significant change being defined as when any parameter exceeded baseline by more than two standard deviations

of the mean throughout the euglycaemic studies, on two or more consecutive occasions. Cognitive function data were analysed by ANOVA with repeated measures design, using data corrected for the initial 40-min euglycaemic period in the hypoglycaemic studies. When there was a statistically significant group-time effect, two-tailed paired *t* tests were used to localise the effects. Simple reaction time, peak hormone and symptom response were analysed by paired Student's *t* test. Results were analysed using SPSS for Windows 10.5 (SPSS, Woking, UK) and differences were regarded as statistically significant if *p* values were less than 0.05.

Results

Insulin and glucose. Insulin infusion raised insulin levels in all clamps, with no significant differences in peak insulin levels between the modafinil and placebo groups in either the euglycaemic studies (148.0 ± 35.3 vs 152.7 ± 35.7 mU/l, $p=0.55$) or the hypoglycaemic studies (156.1 ± 40.8 vs 158.9 ± 26.0 mU/l, $p=0.73$). There were no significant differences in plasma glucose concentrations between the modafinil and placebo groups at euglycaemia (5.0 ± 0.1 vs 5.0 ± 0.1 , $p=0.37$, CV 1.99% and 2.42% respectively) or at any time point during the hypoglycaemic clamps ($p=0.43$; Fig. 1).

Counter-regulatory hormone responses. Cortisol and growth hormone levels decreased during euglycaemic clamp, but concentrations of the other hormones remained stable. All five hormones increased with hypoglycaemia, modafinil having no significant effect either at euglycaemia or at hypoglycaemia. Results are shown in Table 1 and Figure 2.

Heart rate. Heart rate did not change significantly during euglycaemia in either group. During hypoglycaemia, heart rate increased in both groups, but the in-

Fig. 2. Adrenaline (a) and glucagon (b) responses to hypoglycaemia with (open circles) and without (filled squares) modafinil. Data are expressed as means $\pm$ SE

Fig. 3. Change in heart rate during hypoglycaemia with (open circles) and without (filled squares) modafinil. Data are expressed as means $\pm$ SE. § $p < 0.02$, comparing AUC (see methods)

Fig. 4. Change in autonomic symptom scores during hypoglycaemia with (open circles) and without (filled squares) modafinil. Data are expressed as means $\pm$ SE. * $p < 0.05$, comparing total AUC, 60–200 min, see methods; § $p = 0.019$ for AUC, 160–200 min

crease was significantly greater in the modafinil group, where the change was significantly different from both the euglycaemic studies and the hypoglycaemia with placebo studies ($p = 0.016$). Results are shown in Figure 3.

Symptom scores. Total and autonomic symptom scores did not change during euglycaemia in either study. There was a small increase in neuroglycopenic scores, mainly due to an increase in drowsiness, during euglycaemia with placebo, such that the final score was higher than at baseline (6.4 ± 1.0 vs 8.7 ± 2.6 , $p = 0.03$). In the modafinil group there was no difference between baseline and the end of the euglycaemic period (7.2 ± 2.0 vs 7.2 ± 1.5 , $p = 0.17$).

During hypoglycaemia, autonomic and neuroglycopenic symptom scores increased significantly in

both groups compared with euglycaemia. With modafinil there was a significantly greater increase in autonomic symptom responses during the study. As shown in Figure 4, the change in autonomic scores was higher with modafinil between 160 and 200 min, i.e. while plasma glucose was lower than 2.8 mmol/l (AUC $271.3 \pm 118.9/40$ min vs $211.2 \pm 80.4/40$ min, $p = 0.019$). There was no increase in neuroglycopenic symptoms (181.5 ± 258.5 vs $168.6 \pm 226.5/140$ min, $p = 0.94$) at any time.

Cognitive function tests. Modafinil reversed some, but not all, of the deterioration in cognitive performance observed with hypoglycaemia (Table 2). There was no deterioration in performance for any task in modafinil or placebo euglycaemic studies, and modafinil had no significant effect. Performance

Table 1. Hormone and symptom scores during euglycaemic and hypoglycaemic clamps, with and without modafinil

	Euglycaemia		Hypoglycaemia	
	Placebo	Modafinil	Placebo	Modafinil
Adrenaline (nmol/l)				
Basal	0.31±0.29	0.19±0.14	0.13±0.09	0.17±0.09
Peak	0.29±0.08	0.21±0.03	6.38±1.95	6.57±2.62
AUC/140 min	N/A	N/A	2183±77	2117±100
Threshold (mmol/l)	N/A	N/A	3.4±0.3	3.4±0.3
Noradrenaline (nmol/l)				
Basal	1.10±0.41	1.34±0.35	1.11±0.44	1.17±0.41
Peak	1.15±0.30	1.14±0.26	2.50±0.81	2.42±0.76
AUC/140 min	N/A	N/A	505±21	490±22
Threshold (mmol/l)	N/A	N/A	3.2±0.4	3.3±0.4
Growth hormone				
Basal	6.8±8.77	12.53±23.62	7.83±9.61	8.46±13.76
Peak	1.05±0.62	1.5±1.32	87.3±36.3	92.8±33.3
AUC/140 min	N/A	N/A	2755±2467	2972±2825
Threshold (mmol/l)	N/A	N/A	3.3±0.3	3.3±0.4
Cortisol (nmol/l)				
Basal	343.0±165.7	312.7±159	333.7±157.9	323.7±139.2
Peak	246.7±65.14	296.5±86.7	715.7±132.6	738.4±142.1
AUC/140 min	N/A	N/A	14 031±19 633	13 429±14 892
Threshold (mmol/l)	N/A	N/A	3.1±0.4	3.0±0.4
Glucagon (pg/ml)				
Basal	79.3±19.0	86.3±26.6	74.1±13.6	71.8±14.5
Peak	63.7±15.7	63.8±18.1	101.3±32.1	92.2±26.0
AUC/140 min	N/A	N/A	606±765	838±1148
Threshold (mmol/l)	N/A	N/A	2.7±0.7	2.6±0.5
Total symptom score				
Basal	12.8±2.0	14.2±3.9	12.8±1.1	13.0±1.1
Peak	15.6±4.1	14.5±1.9	26.8±8.3	27.8±7.9
AUC/140 min	N/A	N/A	468±356	531±407
Threshold (mmol/l)	N/A	N/A	3.0±0.4	3.1±0.4
Autonomic symptoms				
Basal	6.33±1.0	7.0±2.0	6.33±0.71	6.22±0.44
Peak	6.89±2.03	6.50±0.84	14.22±2.66	15.56±3.09
AUC/140 min	N/A	N/A	299±148	350±180
Threshold (mmol/l)	N/A	N/A	3.1±0.3	3.1±0.4
Neuroglycopenic symptoms				
Basal	6.44±1.01	7.17±2.04	6.44±0.54	6.78±0.83
Peak	8.88±2.60	7.83±1.84	12.56±4.77	12.67±5.17
AUC/140 min	N/A	N/A	169±227	182±259
Threshold (mmol/l)	N/A	N/A	2.6±0.3	2.6±0.3

Data are expressed as means ± SD. N/A, not applicable

in all tasks deteriorated during hypoglycaemia with placebo, but performance in two tasks was better sustained in the presence of modafinil during hypoglycaemia (Stroop colour–word test, $p=0.005$ vs placebo at 2.4 mmol/l; and accuracy of simple reaction task, $p=0.039$ vs placebo at 2.4 mmol/l). For the Stroop colour–word test, only two of the nine sub-

jects studied showed significant deterioration with hypoglycaemia and modafinil, while eight of the nine showed deterioration with hypoglycaemia and placebo. In terms of finger tapping, four-choice reaction time and simple reaction time, modafinil had no significant effect on performance during hypoglycaemia (Fig. 5).

Fig. 5. Cognitive function tests during hypoglycaemia with (open bars) and without (closed bars) modafinil. **a.** Change in number of incorrect scores in Stroop colour-word. **b.** Deterioration in finger tapping score. **c.** Change in four-choice reaction time. **d.** Accuracy of simple reaction time. Data are expressed as means $\pm$ SE. * $p < 0.05$; # $p < 0.05$; § $p < 0.02$

Discussion

In our study, an acute administration of modafinil (2-[diphenylmethylsulphonyl]acetamide) at a low dose had no effect on the counter-regulatory hormone responses to systemic hypoglycaemia, but it increased adrenergic responses (symptoms and heart rate) and also improved some aspects of cognitive performance at low glucose levels, compared with placebo in healthy volunteers.

Modafinil improves vigilance, logical reasoning, short-term memory and tracking tasks in sleep-deprived healthy volunteers and patients with narcolepsy [18, 19, 20]. Its mechanism of action is not completely understood but is believed to work through its action on brain GABA levels. Modafinil lowers brain GABA concen-

tration indirectly by stimulating the noradrenergic system to activate serotonergic neurones, which inhibit the release of GABA [31, 32, 33]. Both the noradrenergic and serotonergic systems are involved in memory processing and cognition [34, 35]. Our finding of reduced drowsiness during the euglycaemic studies are compatible with the known effects of modafinil; and the observed beneficial effects of modafinil on cognitive performance during hypoglycaemia may be related to the activation of both these systems. The observed beneficial effects may also be related to the reduction in GABA concentration or to the activation of another unknown neuronal system [36].

The hypothalamus plays a crucial role in triggering the counter-regulatory response to systemic hypoglycaemia. Hypoglycaemia is associated with an increase in c-fos activity in this region of the brain [37]. A growing body of evidence suggests that the glucose-sensing neurones of the hypothalamus and elsewhere in the brain respond to changes in plasma glucose using mechanisms similar to those used by the pancreatic beta cell [4, 5, 6, 7, 8]. In the latter, a decrease in glucose concentration suppresses insulin secretion. If we postulate that the brain's response to hypogly-

Table 2. Effect of hypoglycaemia and modafinil on cognitive responses

	Euglycaemia		Hypoglycaemia	
	Placebo	Modafinil	Placebo	Modafinil
Stroop colour-word				
Baseline	54.6±12.9	54.8±15.0	53.3±14.1	52.9±12.1
185 min	58.2±12.3	63.0±10.4	38.6±12.7	46.7±9.6 ^a
No. deteriorating	N/A	N/A	8	2
Finger tapping				
Baseline	75.4±10.7	74.3±9.0	75.2±10.0	75.4±8.9
185 min	76.2±10.4	73.6±10.4	65.1±13.3	69.6±10.8
No. deteriorating	N/A	N/A	6	6
Four-choice reaction time (ms)				
Baseline	291±68	281±39	290±56	288±55
185 min	287±61	279±31	427±256	376±167
No. deteriorating	N/A	N/A	5	5
Four-choice reaction accuracy				
Baseline	481±16	488±10	484±12	487±10
185 min	480±15	488±8	473±18	476±19
No. deteriorating	N/A	N/A	7	5
Simple reaction time (ms)				
Baseline	0.58±0.14	0.55±0.11	0.55±0.08	0.59±0.12
185 min	0.54±0.10	0.56±0.10	0.72±0.18	0.70±0.15
No. deteriorating	N/A	N/A	5	2
Simple reaction accuracy				
Baseline	12.0±0	12.0±0	11.88±0.35	11.63±0.52
185 min	11.75±0.71	12.0±0	9.38±3.70	11.15±1.75 ^b
No. deteriorating	N/A	N/A	4	1

Data are expressed as means ± SD. 185 min refers to a plasma glucose of 2.4 mmol/l in the hypoglycaemia study. ^a $p < 0.01$;

^b $p < 0.05$. N/A, not applicable

caemia is activation of sympathetic centres, we must also postulate that any neurotransmitter released by glucose-sensing neurones must be inhibitory of the adrenergic responses, since its release by the glucose-sensing neurone will be stimulated by hyperglycaemia and inhibited by hypoglycaemia, the equivalent of insulin release from the pancreatic beta cell. Hypoglycaemia and agents that close the beta cell K-ATP channel both stimulate neuronal GABA, at least in some brain regions, providing indirect evidence for the above hypothesis [15]. Modafinil, like the state of hypoglycaemia, has been shown to enhance c-fos immunoreactivity within the hypothalamus [38]. At the dose used in our study, modafinil had no effect on any of the counter-regulatory hormones measured, but did increase the heart rate and enhance autonomic symptom response to hypoglycaemia. This suggests increased activation of sympathetic neuronal pathways, or possibly enhanced sensitivity of the adrenergic systems during hypoglycaemia, associated with the actions of modafinil. Neither of these was measured in our protocol.

The effect of modafinil on adrenergic symptoms and heart rate responses during hypoglycaemia in our

studies was not a strong effect. However, this was a study carried out in healthy volunteers, with vigorous normal responses to hypoglycaemia in the control study. Our findings now need to be replicated in patients with diabetes and counter-regulatory impairment. In such subjects, there may be greater scope to show an enhancement of responses, as the control responses will be weaker. Absence of subjective awareness of hypoglycaemia is associated with a three-fold increase in the risk of severe hypoglycaemia [39], and fear of such hypoglycaemia limits patients' attempts to achieve the tight diabetic control that minimises their risk of long-term microvascular complications [40]. An agent that enhances subjective awareness while supporting some aspects of cortical function has the potential to be clinically useful. Modafinil, a medication currently licensed to treat narcolepsy, is worthy of further investigation in this context.

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